3274 Inhalation of 3-Dimensional-(3D)-Printing Fumes Affect the Expression of Markers Associated with Neural Injury

K. Krajnak[1], M. Farcas[1], W. McKinney[1], S. Waugh[1], M. Jackson[1], J. Matheson[2], T. Thomas[2], and Y. Qian[1]. *[1]NIOSH, Morgantown, WV; and [2]Consumer Product Safety Commission, Rockville, MD.*

Inhalation of fumes and particulate generated by 3D-printing with plastics has been shown to induce mild systemic inflammation and oxidative stress. One plastic used for printing is polycarbonate (PC). PC contains organic solvents, bisphenols, and other chemicals that are known neurotoxicants. These neurotoxicants can be released as respirable fumes when PC feed material is heated during printing. The goal of this study was to determine if inhaling fumes and particulate generated when printing with PC resulted in changes in inflammation, oxidative stress and transcripts and proteins involved in the formation of synapses in the central nervous system (CNS). Male Sprague Dawley rats (n = 60, 200-250 g at arrival) were exposed to filtered air or black PC particulate and fumes generated by 3 desktop-3D-printers (exposure 4 h/day (d)). The 4 h average particle concentration delivered to the animals breathing space was 500 µg/m^3. Animals were exposed for 1d or 4 d/week until they had been exposed for 1, 4, 8, 15 or 30 d. The morning after the last exposure animals were euthanized and brain tissue was collected. Tissue from the left side was used for qRT-PCR and tissue from the right side was sectioned and used for immunohistochemistry. Cellular injury (fluorojade staining) was increased in the hippocampus and basal ganglia after 1 day of exposure to 3D fumes. However, staining returned to baseline levels in all groups after 4-30 d of exposure. Glial fibrillary acetic protein staining was also increased in these regions of the brain in 3D-exposed animals on all days of the experiment. In the basal ganglia, there was an increase in the transcription of inflammatory cytokines and anti-oxidant associated genes in 3D fume-exposed animals. Inhalation of 3D printing fumes resulted in an increase in the level of GFAP staining in both the hippocampus and the basal ganglia on all days of the experiment. There were also transient increases in markers of inflammation and oxidative stress. These finding are consistent with studies showing that bisphenol-A (a component of PC) induces oxidative stress and markers of oxidative damage and cellular injury.

3275 Development of Rat DRG Model for Predicting Peripheral Neuroinflammation and Neurotoxicity of Therapeutic Agents

N. Mishra, G. Papadopoulos, L. Friedman, E. Galbreath, Y. Dragan, M. Wagoner, and J. Cohen. *Takeda Pharmaceutical Company Limited, Cambridge, MA.*

Dorsal root ganglion (DRG) toxicity is one of the major concerns for several therapies including adeno-associated viruses (AAVs), oligonucleotides, and chemotherapies. Many AAVs are discontinued due to DRG toxicity but no optimized *in vitro* DRG model is available for predicting DRG or peripheral neurotoxicity of therapeutic agents. In this study, we have developed an *in vitro* DRG model using whole rat DRG cultures incorporating satellite cells, DRG neurons and resident fibroblasts. The neuroinflammatory and neurotoxicity responses are predicted for (i) DRG toxicity, using whole DRG culture and (ii) spinal nerve/root injury, using DRG-microglia co-culture model. Validation of rat DRG-microglia co-culture has been done using (i) inflammatory agents LPS and LPS + IFNy and (ii) marketed tool compounds, paclitaxel and colchicine. Following LPS and IFNy treatments, microglia were activated (as indicated by iNOS induction) and neurites became disorganized. Paclitaxel, which is known to cause peripheral neuropathy in patients, showed disorganization of neurites without any microglial activation. Colchicine, which is known to cause gliosis, showed microglial activation (iNOS induction), and decreased processes dose dependent manner. We have found that this model has utility in detecting oligonucleotide and AAV-mediated toxicities as well. For predicting oligonucleotide mediated DRG toxicity, delivery of oligonucleotide in DRG culture is being optimized and gymnotic method has been chosen as most optimum route of delivery in rat DRG cultures. With AAVs, we've evaluated AAV9 viral vectors expressing GFP for predicting toxicity and efficiency of AAV transduction in DRG culture. Dose dependent increases in AAV transduction, as well as dose dependent increase in cellular death and increased satellite cells activity surrounding DRG neurons have been observed. In conclusion, we have developed a rat DRG model for predicting DRG toxicity and for use as a surrogate to predict spinal nerve injury for therapeutic agents. These models are in the process of further development and validation across drug modalities.

3276 An Investigation of Anxiety-Like Behavior in Male and Female Mice That Were Prenatally Exposed to Endocrine-Disrupting Chemicals (EDCs), Specifically a Mixture of Phthalates

A. Avila Oropeza[1], S. Soriano[2], and M. Mahoney[2]. *[1]California State University San Marcos, San Marcos, CA; and [2]University of Illinois at Urbana-Champaign, Urbana, IL.*

Phthalates are substances added to plastic to make it more flexible and are examples of endocrine-disrupting chemicals. Items that contain phthalates are soaps, shampoos, and cosmetics products. Being exposed to phthalates can cause serious health problems to a person and even types of birth defects. There is various research done on the damages phthalates could cause to the reproductive system but relatively little research on behavior. We conducted a series of tests to research if there are anxiety-like behaviors in mice that were prenatally exposed to an environmentally relevant mixture of phthalates. We treated pregnant CD-1 dams with vehicle (tocopherol-stripped corn oil) or an environmentally relevant phthalate mixture (20, 200 µg/kg/day, and 200 mg/kg/day) daily from gestational day 10.5 to birth. The mixture was composed of 35% diethyl phthalate, 21% di(2-ethyhexyl) phthalate, 15% dibutyl phthalate, 15% diisononyl phthalate, 8% diisobutyl phthalate, and 5% benzylbutyl phthalate. The tests included an open field and a light/dark box test which were then analyzed through video tracking software. The data demonstrated no change of anxiety behavior in mice in the open field test in contrast to the light/dark test which revealed a significant change in transitions between the two zones. More specifically, control animals had fewer transitions than 200 µg or 200 mg group despite showing similar levels of time spent in the light or dark box areas. This then suggests that prenatal exposure to phthalates negatively alters behavior.

3277 Delayed Treatment with Midazolam, Allopregnanolone, and Perampanel Is Superior to Midazolam Alone in Reducing Spontaneous Recurrent Seizures and Neuroinflammation but Does Not Improve Long-Term Behavioral Deficits in the Rat Model of Acute DFP Intoxication

D. A. Bruun, A. Valenzuela, M. Guignet, Y. Tsai, E. Gonzalez, J. Calsbeek, J. Vu, D. Tancredi, D. Harvey, A. Dhir, M. Rogawski, and P. J. Lein. *University of California Davis, Davis, CA.*

Organophosphate (OP) nerve agents are potent neurotoxicants. OPs can trigger seizures that rapidly progress to *status epilepticus* (SE). If not terminated within minutes, OP-induced SE leads to extensive neuropathology and neurological deficits. We have previously shown in a rat model of acute intoxication with the OP diisopropylfluorophosphate (DFP), that treatment with the standard-of care anti-seizure drug midazolam in combination with allopregnanolone (a neurosteroid that acts as a positive allosteric modulator of the $GABA_A$ receptor) and perampanel (a selective non-competitive AMPA receptor antagonist) more effectively terminated acute electrographic seizures than midazolam alone. To determine whether improved seizure control mitigated chronic morbidity, adult male Sprague Dawley rats were dosed with DFP (4 mg/kg sc) immediately followed by atropine sulfate (2 mg/kg im) and 2-PAM (25 mg/ml im). At 40 min post-DFP, rats received midazolam (1.8 mg/kg im), allopregnanolone (6 mg/kg im) and perampanel (2 mg/kg im). Neuropathology was assessed by immunohistochemistry (IHC) at 1, 3, 7 and 28 d post-exposure; behavioral testing was performed at 1 and 2 mo post-exposure. The combined treatment was superior to midazolam alone in reducing spontaneous recurrent seizures (SRS) and neuroinflammation (GFAP and IBA-1 IHC). In contrast, the combined treatment was no better than midazolam in protecting against neurodegeneration (FluoroJade C staining) or in mitigating reactivity (Irwin reactivity test) or cognitive deficits (novel object recognition and contextual fear conditioning). In summary, the combined treatment was effective in improving some but not all long term outcomes, and suggests that neuroinflammatory responses may contribute to OP-induced SRS, but not cognitive deficits. *Supported by NIH CounterACT Program (NS079202).*

3278 Proteomic Analysis of Brain Regions of Adult Long-Evans Rats Exposed to Kainic Acid

E. M. Pitzer[1], G. L. Jung[1], W. T. Padgett[1], W. M. Winnik[1], C. K. Ganta[2,3], N. Clayton[2], H. Jensen[2], R. Herbert[2], and D. W. Herr[1]. *[1]US EPA/CPHEA, Research Triangle Park, NC; [2]NIEHS/NTP, Research Triangle Park, NC; and [3]Integrated Laboratory Systems Inc., Research Triangle Park, NC.*

We are using proteomics to measure changes in protein signatures produced by prototypical neurotoxicants, allowing better understanding of the molecular events produced by these compounds. Adult male Long Evans rats were treated with kainic acid (KA; 6 mg/mL, s.c., chosen not to produce myoclonic seizures), vehicle (VC; buffered saline, 0 mg/mL), or were non-injected (cage controls, CC). At 3 or 24h post-exposure, the rats were perfused with DPBS and brain regions collected and stored at -80°C. Hippocampal samples were assessed for proteomic content using Orbitrap LC-MS, and proteins were identified and processed using Proteome Discover. Pathway analysis was performed using Ingenuity Pathway Analysis (IPA) software (differential protein cutoff: ≤ log2(0.8), ≥ log2(1.2), FDR p-value ≤ 0.05). A total of 1894 and 1770 proteins were identified for mapping with IPA, at 3 and 24 h respectively. Of those proteins, 199 and 103 were altered by KA treatment at 3 and 24h, respectively. At 24h the top proteomic inhibited pathways included synaptogenesis signaling pathway, G Beta Gamma signaling, and Ephrin receptor signaling. Activated pathways at 24h included PTEN signaling, semaphoring neuronal repulsive signaling pathway, and autophagy. Proteins impacted in these pathways included, RAC (Rho family)-alpha serine/threonine-protein kinase (AKT; downregulated, in all above pathways), Ras GTPase (RAS; downregulated, in all above pathways except autophagy), and Growth factor receptor-bound protein 2 (GRB2; upregulated, pathways: synaptogenesis signaling, G Beta Gamma, ephrin receptor, PTEN), as well as additional up- or downregulated proteins. An additional set of rats were treated and sacrificed at 3, 24, or 48h following KA exposure (0 or 6

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